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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF GABAPENTIN IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A validated simple, sensitive, specific and precise RP-HPLC method was developed for the determination of Gabapentin in pure and pharmaceutical formulations. Method was carried on Zodiac C_{18} column (250 mm × 4.6 mm × 5 µ particle size) using Methanol : Acetonitrile : Triethylamine (50 : 25 : 25) as mobile phase. Detection was carried out by U.V. at 211 nm. The proposed method obeyed linearity in the range of 10-60 µg/mL. and met all specifications as per ICH guidelines. Statistical analysis revealed that this method can be used in routine quality control studies of Gabapentin in pure and its formulations.

Keywords: Gabapentin, C_{18} column, Reverse phase, Validation, Spectificity.

INTRODUCTION

Gabapentin[1,2] chemically 1-(amino methyl)-cyclohexaneacetic acid is a cyclic GABA analogue, used as a anti-convulsant drug, effective in prevention of frequent migraine headache. It is of white in colour, highly soluble in water.

Literature survey reveals several methods were reported for determination of gabapentin in biological fluids and in dosage forms by U.V.[3,10], HPLC[4], LC-MS[5,6], GC-MS[7], Capillary electrophoresis[8,11] and HPTLC[9]. In the present study, a new RP-HPLC method was developed, which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines.

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Instrumentation

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Gabapentin an isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5 µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC-7000 UV-detector. A 20 µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Standards and chemicals used

Gabapentin was provided by Dr. Reddy's laboratories. All the chemicals Acetonitrile, methanol, water, Triethylamine were HPLC grade, Merck Specialties Private Limited, Mumbai, India.

Commercial tablets of Gabapentin were purchased from local market.

Preparation of the mobile phase

Into a 1000 mL cleaned volumetric flask, HPLC grade methanol 500 mL, acetonitrile 250 mL and Triethylamine 250 mL (which are filtered through 0.25 mm membrane filters by vacuum filtration) were slowly added, mixed well and sonicated upto 20 min. Cool the above solution and pH was adjusted to 5.8 with orthophosphoric acid. This solution is again sonicated to 10 min. Cool the solution to room temperature and use for chromatography method.

Preparation of standard drug solutions

100 mg of Gabapentin was accurately weighed and is dissolved in few mL of the mobile phase and sonicated for few min to dissolve the drug completely. Then it is filtered through 0.2 µ ultipore filter paper and the volume is made upto 100 mL with mobile phase to get a concentration of 1 mg/mL (free base) stock solution. This solution is further diluted with same solvent to obtain required working standard concentrations.

Sample preparation

20 commercial tablets of Gabapentin were finely powdered and the powder equivalent to 50 mg of gabapentin accurately weighed to 50 mL volumetric flask and dissolved in few mL of mobile phase. The above solution was subjected to sonication for 15 min. after getting clear solution it is filtered through 0.25 µm membrane filters and the solution is made upto 50 mL with mobile phase resulting in preparation of 1 mg/mL solution.

This is further diluted so as to obtain required concentration of Gabapentin pharmaceutical dosage form.

Methodology

The HPLC system was stabilized for thirty min. by passing mobile phase, detector was set at 211 nm, flow rate of 1.0 mL/min to get a stable base line. One blank followed by six replicates of a single standard solution was injected to check the system suitability. Six replicates of each standard solutions 10, 20, 30, 40, 50 and 60 µg/mL were injected. Calibration graph was plotted by concentration of Gabapentin on X-axis and peak area on Y-axis. The amount of drug present in sample was computed in calibration graph.

Pharmaceutical formulations

Prepared dilution of pharmaceutical formulation is injected and the procedure described under bulk samples was followed. The amount of drug present in sample was computed in calibration graph.

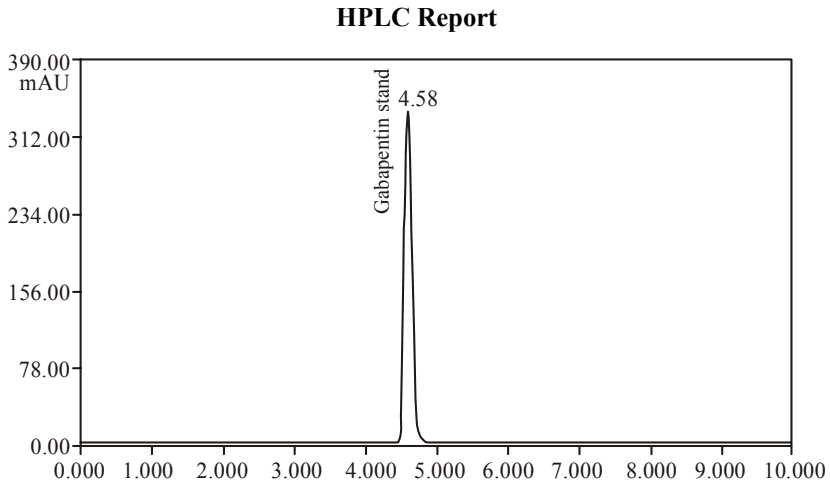
Table 1: Optimized chromatographic conditions for estimation of Gabapentin

Parameter	Condition
Mobile phase	Methanol : ACN : TEA (50 : 25 : 25) (v/v/v)
Type of elution	Isocratic
pH	5.8
Detection wavelength	By U.V. at 211 nm
Column	Zodiac C_{18} column (250 x 4.6 mm, 5 µ)
Column temp.	Ambient
Injection volume	20 µL
Flow rate	1.0 mL/min.
Run time	10 min.
Retention time	4.58 min.

RESULTS AND DISCUSSION

The objective of the present work was to develop simple, precise and reliable HPLC method for the analysis of Gabapentin in bulk and pharmaceutical dosage form. This is

achieved by using the most commonly employed column C_{18} with U.V. detection at 211 nm. The representative chromatogram indicating Gabapentin is shown in Fig. 1.



ID	Name	Retain T	Height	Area	Conc.	Tail factor	Theo. plate
1	Gabapentin Standard Sum	4.583	33658	244676.3	100.000	1.31	7923
			33658	244676.3	100.0000		

Fig. 1: Chromatogram of Gabapentin

Parameter fixation

In developing this method, a systemic study of effects of various parameters was undertaken by varying one parameter at a time and controlling all other parameters. The following studies were conducted for this purpose.

Stationary phase characteristics

Based on nature and solubility characteristics of gabapentin, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried C_{18} column was found to be optimum.

Mobile phase characteristics

In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents

like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of Methanol : ACN : TEA (50 : 25 : 25) (v/v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Validation of the proposed method

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

Linearity

Linearity was performed by preparing standard solutions of Gabapentin at different concentration levels, twenty micro liters of each concentration was injected in into the HPLC system. The peak responses were read at 211 nm and the corresponding chromatograms were recorded. Linearity plots of concentration over areas were constructed individually. Linearity results were obtained in the concentration range of 10-60 µg/mL. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient values and the results were presented in Table 2.

Table 2: Linearity results of Gabapentin

Concentration (µg/mL)	Peak area
10	91926
20	172855
30	244676
40	317468
50	399191
60	481152

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as intraday precision, Inter day precision.

Intra day precision

To study the intra day precision, six replicate standard solutions (30 µg/mL) of Gabapentin was injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.96, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 3.

Table 3: Intra day precision vlues

Sample	Conc. (in ppm)	Injection No.	Peak areas	RSD (Acceptance criteria ≤ 2.0%)
Gabapentin	20	1	244676	0.96
		2	241923	
		3	246782	
		4	243921	
		5	242016	
		6	246296	

Inter day precision

To study the inter day precision, six replicate standard solutions (30 ppm) of Gabapentin was injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.811, which are well within the acceptable criteria of not more than 2.0.

Specificity

The effect of wide range of excipients and other additives usually present in the formulation of Gabapentin in the determinations under optimum conditions were investigated infact, may have no observation at this UV Maximum. Chromatographic parameters maintained are specific for gabapentin

Ruggedness

Percent recoveries of gabapentin was good under most conditions and didn’t show any significant change, when the critical parameters were modified.

Table 4: Ruggedness values

Sample	Conc. (in ppm)	Injection No.	Peak areas	RSD (Acceptance criteria ≤ 2.0%)
Gabapentin	20	1	255276	1.53
		2	257254	
		3	251972	
		4	253284	
		5	258697	
		6	256061	

Table 5: Limit of detection and limit of quantification for Gabapentin

Parameter	Values
Limit of quantification	2.5 µg/mL
Limit of detection	0.75 µg/mL

Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of 20 ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table 6. Satisfactory recoveries ranging from 98% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate.

Table 6: Recovery results

Level	Concentration (µg/mL)	Amount of Gabapentin spiked (µg/mL)	Total in ppm	Amount of Gabapentin recovered (ppm)	% Recovery
50%	20	10	30	29.68881	98.96271
	20	10	30	30.45844	101.5281
	20	10	30	29.88266	99.60887

Cont…

Level	Concentration (µg/mL)	Amount of Gabapentin spiked (µg/mL)	Total in ppm	Amount of Gabapentin recovered (ppm)	% Recovery
100%	20	20	40	39.71273	99.28182
	20	20	40	40.18874	100.4719
	20	20	40	39.94784	99.86959
150%	20	30	50	50.57591	101.1518
	20	30	50	50.75891	101.5178
	20	30	50	50.70931	101.4186

Robustness

The robustness study was performed by slight modification in flow rate of the mobile phase, pH of the buffer and composition of the mobile phase. Gabapentin at 2 ppm concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature.

Formulation

S. No.	Brand Name	Concentration	Area	Amount found	% Assay
1	Neurontin - 600 mg	30 ppm	241933	29.66	98.87

REFERENCES

1. The Martindale 35th Edition, The Complete Drug Reference, Lambeta High Street, London (2006) pp. 362-363.
2. United States of Pharmacopoeia, 30, NF25, **32(6)** (2007) p. 1689.
3. H. E. Abdellatef and H. M. Khalil, Colorimetric Determination of Gabapentin in Pharmaceutical Formulation, J. Pharma. and Biomed. Anal, **31(1)**, 209-214 (2003).
4. A. B. Ciavarella, A. Vilayat et al., Development and Application of a Validated HPLC Method for the Determination of Gabapentin and its Major Degradation Impurity in Drug Products, J. Pharma. and Biomed. Anal., **43**, 1647-1653 (2007).

5. A. Ojha, R. Rathod, C. Patel and H. Padh, LC-MS Determination of Gabapentin from Human Plasma, Chromatographia, **66**, 853-857 (2007).

6. M. Nomura, Y. Sakamoto, N. Nagashima, N. Takehara and K. Kitamura, Determination of Methylcobalamin in Serum by Liquid Chromatography-mass Spectrometry with Selected Ion Monitoring, Boil. Psychiatry, **42**, 43 (1997).

7. M. Pujadas, S. Pichini, Civite, E. Santamarina, K. Perez, Rafel D. Torre, A Simple and Reliable Procedure for the Determination of the Psychoactive Drugs in Oral Fluid by Gaschromatography-Mass Spectrometry, J. Pharma. and Biomed. Anal., **4**, 594-601 (2007).

8. B. R. Bharat, A. Shantha, P. Malairajan and S. Anbazhagan, Simulatneous Estimation and Validation of Gabapentin and Methylcobalmin in Combined Dosage form by RP-HPLC, Indian Drugs, **44(10)**, 784-788 (2007).

9. R. T. Sane, U. Pendse, A. Moghe, S. Khedkar and P. Patil, Gabapentin in Pharmaceutical Preparation by HPTLC, Indian Drugs, **40(9)**, 547-548 (2003).

10. Varsha R. Galande, K. G. Baheti and M. H. Dehgham, UV-Visible Spectrophotometric Method for Estimation of Gabapentin and Methyl Cobalamin in Bulk and Tablet, Int. J. Chem. Tech. Res., **2(1)**, 695-699 (2010).

11. S. Y. Chang and F. Y. Wang, Determination of Gabapentin in Human Plasma by Capillary Electrophoresis with Laser Induced Fluorescence Detection and Acetonitrile Stacking Technique, J. Chromatography B: Biomed. Appl., **799**, 265-270 (2004).

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